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Nonimmune Fetal Hydrops and Placentomegaly: Diagnosis of Familial Wiedemann-Beckwith Syndrome With Trisomy 11p15 Using FISH

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We have studied a family in which four members of the same generation were affected with Wiedemann-Beckwith syndrome (WBS). Trisomy 11p15 was demonstrated using molecular probes in interphase nuclei of formalin-fixed paraffin-embedded placenta from a stillborn fetus and in peripheral blood lymphocytes from two liveborn female relatives. Clinical examination showed nonimmune hydrops and placentomegaly in two siblings and multiple phenotypic abnormalities consistent with WBS in the two other relatives. Paternal karyotype of the stillborn infants demonstrated a reciprocal translocation (46,XY,t(10;11)(q26;p15)) explaining the origin of the extra 11p15 material. This study illustrates the advantages of FISH for interphase analysis of chromosome aberrations otherwise not detected even by conventional cytogenetic analysis and documents that nonimmune hydrops associated with placentomegaly may be the presenting features in familial WBS.

KEY WORDS: FISH, nonimmune hydrops, trisomy 11p15, placentomegaly, Wiedemann-Beckwith syndrome

INTRODUCTION

Wiedemann-Beckwith syndrome (WBS) is characterized by exomphalos, macroglossia, gigantism, ear lobe grooves and/or pits, visceromegaly, hemihypertrophy and neonatal hypoglycemia, and a tendency to develop embryonal tumors. Most of the reported cases have occurred sporadically and less than 30 kindreds with WBS have been published with two or more affected subjects [Viljoen and Ramesar, 1992]. The diagnosis is generally based on clinical and pathologic findings and is made at birth or in early childhood. In most cases there are no visible chromosome anomalies but current data show that the syndrome can be caused by paternal duplication of 11p15.5 or by paternal disomy [Little et al., 1991].

This report describes a family in which four relatives of the same generation were affected with WBS. In three cases, trisomy 11p15 was demonstrated using FISH.

CLINICAL REPORTS

Patient 1

The mother was a 28-year-old woman in her second pregnancy. In the 28th week of gestation, ultrasonography showed polyhydramnios and placentomegaly, and the patient underwent elective termination. Amniotic alpha-fetoprotein was normal for gestational age. Chromosomes of amniocentesis were apparently normal (46,XX). The female fetus weighed 1,450 g and had a crown–rump length of 40 cm, generalized hydrops, macroglossia, right adrenomegaly and nephromegaly (Fig. 1). Microscopic study documented renal medullary dysplasia. Cytomegaly of adrenal cells in the fetal cortex was also evident in spite of autolysis. The placenta was large and pale and weighed 1,200 g. A 15 × 14 × 6 cm, reddish, multinodular mass extended from the subchorionic plate protruding in the maternal surface (Fig. 2). Placental villi showed markedly edematous stroma, scalloped borders and occasional trophoblastic inclusions. The mass corresponded to a proliferation of tightly arranged capillary vessels of a large chorioangioma. FISH was performed in paraffin-embedded tissue from the placenta using the alpha-satellite chromosome 11 and 11p15.5-pter-specific probes according to the manufacturer's protocol (Oncor, Gaithersburg, MD) and evaluated following the criteria established by Hopman et al. [1990]. Results are interpreted as significant for polysomy when over 5% of the nuclei had more than two signals for the specific chromosome [Lee et al., 1993]. Two spots were found in 42% of the trophoblastic and stromal nuclei with the probe for the 11 centromere region, whereas 36% of the nuclei showed 3 fluorescent signals for the 11p15.5-pter probe. Thus, there was disomy of the 11 centromere and trisomy of 11p15.5 (Fig. 3).

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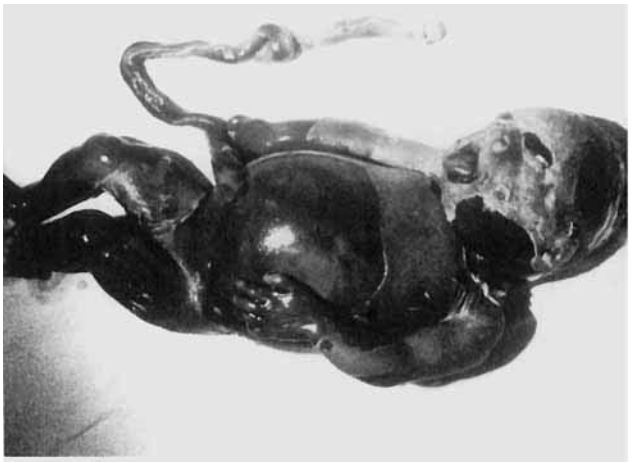

Fig. 1. Hydropic fetus with macroglossia (patient 1).

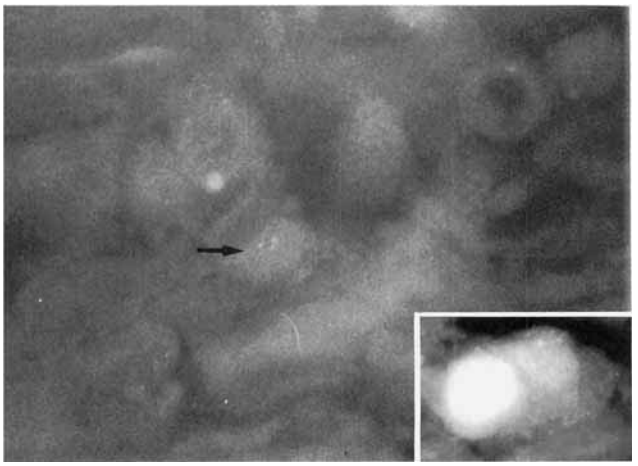

Fig. 3. Three fluorescent signals are visible in interphase nuclei (arrow). FISH in formalin-fixed paraffin-embedded placenta with the probe for 11p15. **Inset:** Two fluorescent signals in interphase nuclei. Same technique with the probe for the chromosome 11 centromere (patient 1).

Both parents were phenotypically normal. The parents had been analyzed after losing their first pregnancy. The mother had had a normal karyotype while the father's had been abnormal, with reciprocal translocation between the long arm of chromosome 10 and the short arm of chromosome 11: 46,XY,t(10;11)(q26;p15).

Patient 2

This female stillborn fetus was born to the same parents 2 years previously. Pregnancy termination was due to polyhydramnios at 23 weeks of gestation. The fetus showed a distended abdomen with ascites and left nephromegaly with renal vein thrombosis. There was cytomegaly in the fetal cortex. The placenta was large and heavy for gestational age (420 g). Microscopic examination showed hydropic villi with irregular borders and rare trophoblastic inclusions.

Patient 3

This 1-year-old girl was born after 35 weeks of gestation. A 4-year-old brother was healthy. Delivery was normal. Birth weight was 3,150 g and length was 49 cm (>95th percentile). Her father and that of patients 1 and 2 were brothers.

The infant had a prominent forehead, downslanting palpebral fissures, epicanthal folds, wide and flat nasal bridge, hypertelorism, strabismus, weight >90th centile (12.8 kg), widely spaced nipples, right hemi-hypertrophy, hypertrophy of upper and lower limbs, macroglossia, malocclusion of teeth, mental retardation, seizures with abnormal EEG, and small umbilical hernia. X-ray films showed advanced bone age and abdominal ultrasonography demonstrated urinary tract anomaly. Karyotype analysis did not show chromosome aberration. FISH in interphase nuclei and metaphases from peripheral blood lymphocytes for the 11p15.5-pter probe study demonstrated trisomy in 73% of the cells (Fig. 4). Her father refused to have a chromosome analysis but we interpret that he must have been the carrier of the translocation.

Fig. 2. Chorioangiomatous area to the left contrasting with pale areas of the remaining placenta (patient 1).

Patient 4

This 4-year-old girl was the daughter of a cousin of the paternal family of patients 1, 2 and 3. Her weight was 20 kg (>90th percentile). Birth weight was 3,900 g. Facial and body anomalies were similar to those described for patient 3 except for the urinary tract anomaly. Chromosomes were apparently normal. FISH in interphase nuclei and metaphase from peripheral blood lymphocytes for the 11p15.5-pter probe study showed trisomy in 80% of the cells. Fluorescent signals were visualized on both chromatids of both 11 homologues and on both chromatids of one 10q (Fig. 5).

DISCUSSION

Patients 1 and 2 had fetal and placental findings consistent with WBS. Placentomegaly and nonimmune hydrops in the WBS were reported by Lage [1991]. Placental chrioangioma in WBS was reported previously by us [Drut et al., 1992a] and interpreted as part of the

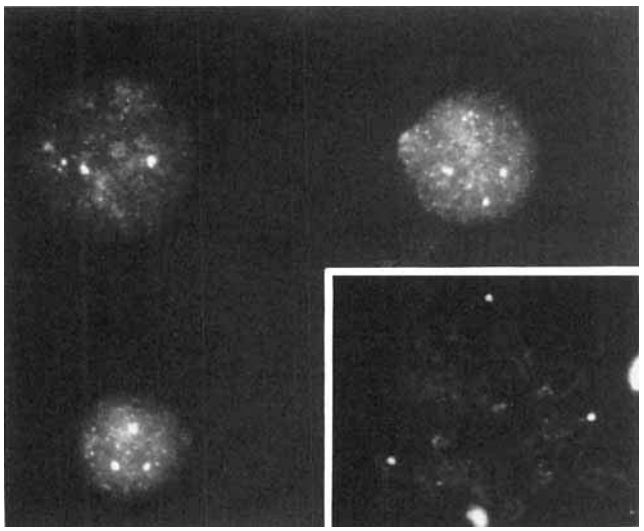

Fig. 4. Three fluorescent signals are highlighted in a metaphase and in interphase nuclei of peripheral blood lymphocytes. FISH with the probe for 11p15 (patient 3). Inset: same in another area of the smear.

spectrum of the syndrome. Patients 3 and 4 had manifestations compatible with WBS.

The association between trisomy 11p15 and WBS is well established [Waziri et al., 1983; Turleau et al., 1984; Journel et al., 1985]. In addition, other rare chromosome abnormalities have also been described [Haas et al., 1986]. Chromosome analysis of amniotic fluid in patient 1 and peripheral blood lymphocyte culture in patients 3 and 4 failed to demonstrate visible chromosomal anomalies, probably due to lack of high resolution banding. In patient 1 the diagnosis of WBS with trisomy 11p15-pter was made on paraffin sections of placental tissue and in patients 3 and 4 on metaphases and on interphase nuclei of peripheral blood lymphocytes by FISH. Since the paternal karyotype of the stillborn infants showed a reciprocal translocation (46,XY,t(10;11)(q26;p15)) we assume that the patients had inherited the der(10) chromosome from the father and one normal chromosome 11 from each parent and thus, were partially trisomic for 11p15. The in situ method proved to be useful for routinely processed pathological samples when conventional cytogenetic techniques are not feasible or available. However, since this method is limited by the specific probe to be used, it is necessary to make an appropriate selection based on clinico-pathological findings.

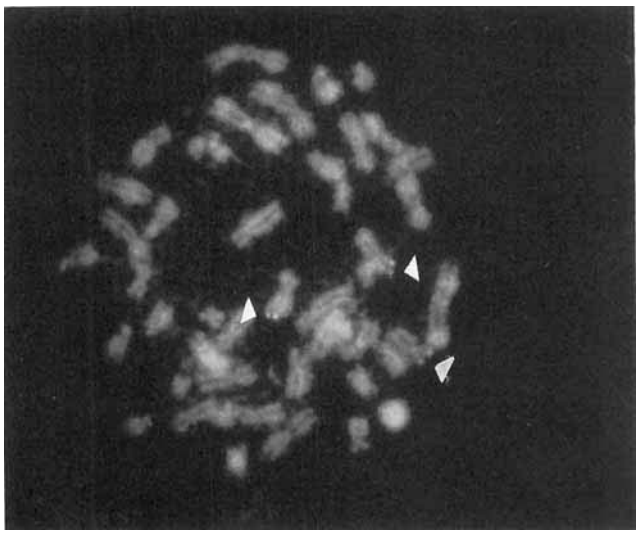

Fig. 5. Three pairs of fluorescent signals (arrowheads) in a metaphase of peripheral blood lymphocytes. FISH with the probe for 11p15 (patient 4).

The low frequency of nuclei showing three hybridization signals in the placenta (36%) compared to the peripheral blood cultured lymphocytes (73% and 80%) can be explained by a reduced penetration of the probes or antibodies into the nuclei or to the fact that since not all the tissue sections include the complete nucleus in many cells, the target DNAs do not share the same plane [Drut et al., 1992b].

Although most cases of WBS are sporadic, familial cases have been reported [Pettenati et al., 1986; Viljoen and Ramesar, 1992]. Despite the family history documenting close relatives with the same disorder, the present family was not known to be affected by WBS until the stillborn infants were studied. Figure 6 shows the pedigree of the family. In this pedigree the WBS apparently was transmitted horizontally by unaffected parents who had affected daughters. At least 27 kindreds with WBS have been published where two or more relatives were affected, with different interpretations of the mode of inheritance [Viljoen and Ramesar, 1992].

Fetal overgrowth as well as the development of tumors observed in the WBS have been related to the 11p15.5 region of human chromosome 11 that harbors the genes coding for the globins, insulin and oncogen $HRAS_1$. Henry et al. [1991] studied, by RFLP analysis, 6 loci from 11pter to 11p15.5, demonstrating uniparental disomy for INS-IGF2 to $HRAS_1$ and to HBBP in 3 BWS patients with cytogenetically normal chromosome 11. In addition the observations that IGF2 is selectively expressed from paternally inherited allele in normal fetal tissue [Ogawa et al., 1993] and in WBS patient tissues [Ohlsson et al., 1993] further support that IGF2 may contribute to the WBS phenotype. In cases with a duplication of 11p15.5 the parental origin is usually paternal [Henry et al., 1989; Brown et al., 1990, 1992; Mannens et al., 1991; Moutou et al., 1992]. Genomic imprinting has been implicated as a likely explanation in the irregular inheritance pattern of the syndrome [Koufos et al., 1989; Brown et al., 1990, 1992; Reik, 1989; Moore and Haig, 1991]. According to Little et al. [1991], duplication of the active paternal allele, whether by 11p15 trisomy or paternal disomy could explain the parental imprinting. The same authors suggest that the disease could also arise if the maternal allele is inappropriately activated. While presenting data of DNA polymorphism analyses from WBS patients, Kubota et al. [1994] concluded that excess functional copy of allele at chromosomal region 11p15 may cause WBS and proposed a 5-category classification of the syndrome.

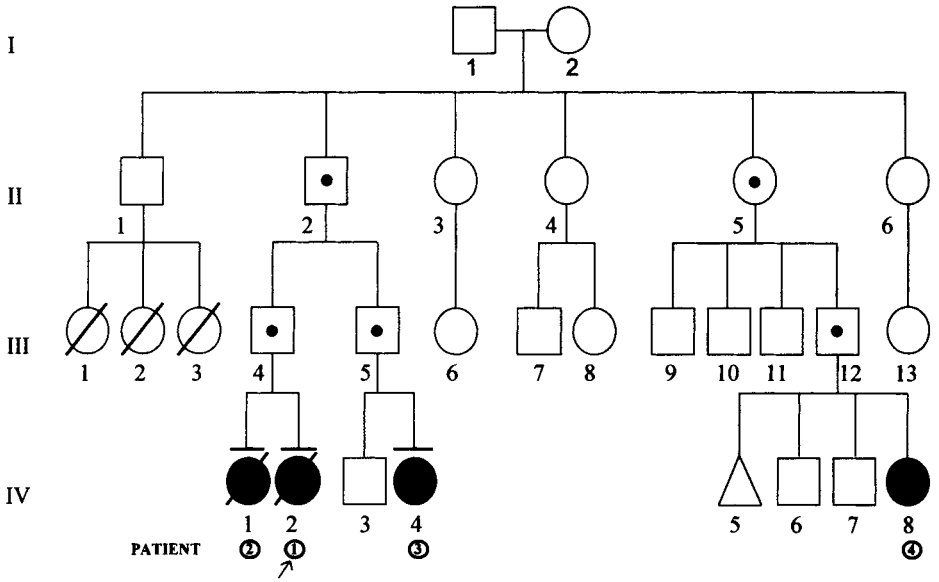

Fig. 6. Pedigree of the family segregating BWS.

In view that the father of patients 1 and 2 is a t(10;11)(q26;p15) carrier, the risk of disease in the patient's sib is of clinical significance for either trisomy 11p15 or monosomy 10q22.

Trisomy 11p15 may now be detected by FISH in cultures of chorionic villi samples, amniotic fluid and peripheral blood lymphocytes, or even in formalin-fixed, paraffin-embedded tissues, prenatally or at birth, helping to understand the relationships between chromosomal abnormalities and pathological findings.

An accurate diagnosis will allow antenatal diagnosis of possible affected fetuses, a correct clinical interpretation of relatives, appropriate genetic counseling and follow-up of those members at risk of developing tumors.

Nonimmune hydrops fetalis associated with placentomegaly should be included in the list of presenting features of WBS.

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